

An Investigation of the Influence of Chain Length on the Interfacial Ordering of L-Lysine and L-Proline and Their Homopeptides at Hydrophobic and Hydrophilic Interfaces Studied by Sum Frequency Generation and Quartz Crystal Microbalance

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Abstract

Sum frequency generation vibrational spectroscopy (SFG) and quartz crystal microbalance with dissipation monitoring (QCM-D) are employed to study the interfacial structure and adsorbed amount of the amino acids L-lysine and L-proline and their corresponding homopeptides, poly-L-lysine and poly-L-proline, at two liquid-solid interfaces. SFG and QCM-D experiments of these molecules are carried out at the interface between phosphate buffered saline at pH 7.4 (PBS buffer) and the hydrophobic deuterated polystyrene (d_8 -PS) surface as well as the interface between PBS buffer and hydrophilic fused silica (SiO_2). The SFG spectra of the amino acids studied here are qualitatively similar to their corresponding homopeptides; however, SFG signal from amino acids at the solid/PBS buffer interface is smaller in magnitude relative to their more massive homopeptides at the concentrations studied here. Substantial differences are observed in SFG spectra for each species between the hydrophobic d_8 -PS and the hydrophilic SiO_2 liquid-solid interfaces, suggesting surface-specific different interfacial ordering of the biomolecules. At the solution concentrations studied here, QCM-D measurements also indicate that on both surfaces poly-L-lysine adsorbs to a greater extent than its constituent amino acid L-lysine. The opposite trend is demonstrated by poly-L-proline which sticks to both surfaces less extensively than its corresponding amino acid, L-proline. Both of these trends are explained by differences between the amino acids and their corresponding homopeptides in charge density, molecular mass, and solution concentrations. Additionally, we find that the adsorption of the molecules studied here can have a strong influence on interfacial water structure as detected in the SFG spectra.

Introduction

The study of biomolecules, especially proteins and peptides, at interfaces remains an active area of study for both the surface science and biomedical communities.¹⁻³ In recent years, the surface-specific technique of Sum Frequency Generation (SFG) Vibrational Spectroscopy has been applied to the study of adsorbed proteins and peptides at the solid-liquid interface.⁴⁻⁸ Of the several interfaces studied in the literature, two stand out as being quite difficult to study: biomolecules at the silica (SiO₂)/liquid interface and amino acids (at physiological pH) at any solid/liquid interface.⁵ These two systems have proven challenging due to the fact that C-H modes were not observed in the SFG spectra. The cause of these phenomena is not known *a priori*, but postulated to result from absence of amino acid side chain ordering. In the case of biomolecules at the silica/buffer interface, both the solvent and the surface are hydrophilic while a peptide or protein contains side chains that generally are more hydrophobic. In the past it appeared that these hydrophobic side chains lacked sufficient driving force to order at the hydrophilic silica/buffer interface to the extent necessary to be observable via SFG. This has been demonstrated for various chain-lengths of biomolecules: amino acids, small and longer peptides, and large proteins.^{5,9-11} Amino acids, the individual building blocks of proteins, have been studied with SFG before by Watry and Richmond,¹² who examined several amino acids at the oil/water interface; Ji and Shen,¹³ who quantitatively studied leucine at the air/water interface; Kim et al.,¹⁴ who studied phenylalanine at the glassy carbon electrode; and most recently Paszti et al., who examined several different amino acids adsorbed on different hydrophilic solids.¹⁵ In the case of amino-acids at the hydrophobic solid/liquid interface, no C-H mode ordering has been observed in the literature (with the

exception of electrochemical interfaces¹⁴). The reason for this is unclear; ordered C-H modes of proteins and peptides have been observed at the hydrophobic solid/buffer interface previously.

In this report, we experimentally show C-H ordering in the SFG spectrum of lysine amino acid and proline amino acid at the deuterated polystyrene (*d*₈-PS)/phosphate buffer saline (PBS buffer) interface; additionally, we present a SFG spectrum demonstrating C-H ordering of poly-L-lysine at the silica (SiO₂)/PBS buffer interface. Next, we discuss the differences in the adsorbates' interfacial structure on both hydrophilic and hydrophobic surfaces, a phenomenon that was first observed with SFG by the Cremer group.¹¹ We also compare how chain length influences the interfacial structure of lysine and proline peptides at both hydrophobic and hydrophilic surfaces. Additionally, we compare the interfacial structure of homopeptides to amphiphilic peptides adsorbed to both hydrophobic and hydrophilic surfaces. Finally, we discuss the influence of an adsorbed biomolecule on the ordering of interfacial water molecules.

Experimental

Chemicals. All molecules were dissolved in pH 7.4 PBS buffer from Sigma-Aldrich (cat. no. P-5368). L-Lysine amino acid was obtained from Sigma-Aldrich (cat. no. L5501) and poly-L-lysine was also obtained from Sigma-Aldrich (cat. no. P2658). The concentration of L-lysine used in these experiments was 16.5 mg/mL and the concentration of poly-L-lysine was 12.5 mg/mL. L-Proline amino acid was obtained from Sigma-Aldrich (cat. no. P5607) and poly-L-proline was also obtained from Sigma-Aldrich (cat. no. P2254). The concentration of L-proline used was 500 mg/mL and the concentration of poly-L-proline was 0.5 mg/mL. All concentrations were chosen to

maximize SFG signal while still maintaining solubility. In all cases the monomeric solution concentration of side chains was higher for the amino acid than the polypeptide. All chemicals were used as received.

SFG. The theory of SFG has been described elsewhere.¹⁶⁻²⁰ Due to the small SFG signal from amino acids at the solid/liquid interface, and biomolecules at the SiO₂/liquid interface, we have chosen a different experimental geometry than our previous experiments.¹⁷ The angle of incidence of the visible was 57° relative to the surface normal, and the angle of incidence of the infrared was 64° relative to the surface normal. The influence of the choice of this geometry has been discussed in detail elsewhere, and will be discussed here only briefly.¹⁹ By conducting experiments at these angles, it became possible to detect individual amino acids with the signal to noise ratio necessary to observe their vibrational modes at the solid-liquid interface. These angles were chosen since they are close to the total internal reflection angle, but not actually at this angle. SFG signal intensity depends both on the square of the number density and orientational ordering of interfacial molecules.^{10,17,21} All spectra presented here are in the $S_{\text{SFG}}S_{\text{VIS}}P_{\text{IR}}$ polarization combination.

QCM. Detailed descriptions of the background and theory of quartz crystal microbalance with dissipation monitoring have been given elsewhere.²²⁻²⁴ All QCM-D measurements were conducted using a Q-Sense D300.²⁵ The SiO₂ coated QCM sensor crystals used were purchased directly from Q-Sense (Glen Burnie, MD). The *d*₈-PS coated crystals were prepared from gold coated sensor crystals (purchased directly from Q-sense) by spin coating *d*₈-polystyrene (2% wt) in toluene (3000 rpm, 45 sec) and subsequently annealing for 12 hours at 120 °C. Resulting film thicknesses were

previously determined to have a thickness of ~100 nm by ellipsometry. The fundamental, 3rd, 5th, and 7th overtone frequencies for each crystal were determined in pure PBS solution at pH 7.4 immediately prior to introduction of amino acid and peptide solutions. Surface concentrations were determined using the Sauerbrey equation²⁶ (which linearly correlates a change in frequency with an adsorbed mass), Δ frequency data from the 3rd overtone, and a mass sensitivity constant C of $17.7 \text{ ng} \cdot \text{cm}^{-2} \cdot \text{Hz}^{-1}$ at 5 MHz. During measurement, sensor crystals and solutions were maintained at 20.3 ± 0.2 °C. After establishing a stable baseline in buffer the sensor crystal surfaces were exposed to biomolecule solutions at concentrations matching those used in SFG experiments. Minor linear instrument drift was present in all QCM-D measurements. This was corrected for by conducting a linear regression of the 10-30 minutes of 3rd overtone frequency data collected from sensor crystals in pure PBS immediately prior to introduction of biomolecule solutions for each individual measurement. The resulting linear equation was then used to correct experimental data for the linear instrument drift for each individual measurement. The QCM instrument cell was cleaned between measurements with 2% Hellmanex II detergent solution (Hellma GmbH & Co. Mullheim, Germany). After measurement, d_8 -PS coated Au sensor crystals were rinsed consecutively with toluene and acetone prior to O₂ plasma treatment. SiO₂ sensor crystals were directly cleaned by O₂ plasma treatment.

Results

SFG. The SFG spectrum of poly-L-lysine on d_8 -PS is shown in Figure 1 (a). There are two peaks apparent in the SFG spectra: 2870 cm^{-1} assigned to CH₂ (s), and 2935 cm^{-1} assigned to CH₂ (as).¹² These peaks are also apparent in the SFG spectrum of

lysine on d_8 -PS with much weaker intensity (Figure 1 (b)); (it should be noted here that the 2870 cm^{-1} mode is very weak and is just above the level of the background). The SFG spectrum (Figure 2 (a)) of poly-L-proline on d_8 -PS shows three intense modes at 2875 cm^{-1} , 2935 cm^{-1} , and 2980 cm^{-1} . The mode at 2875 cm^{-1} is assigned to a combination of, C_βH_2 (s), $\text{C}_\gamma\text{H}_2$ (s), and $\text{C}_\delta\text{H}_2$ (s) (see Figure 2 (a) for details on the nomenclature).²⁷ The mode at 2935 cm^{-1} is assigned to a combination of C_βH_2 (s) and $\text{C}_\gamma\text{H}_2$ (s).²⁷ Finally the vibration at 2980 cm^{-1} is assigned to a combination of C_βH_2 (as) and $\text{C}_\gamma\text{H}_2$ (as).²⁷ These modes are also clearly observed in the SFG spectra of L-proline on d_8 -PS (Figure 2 (b)), albeit with much less intensity than the poly-L-proline homopeptide.

The interfacial water structure at the d_8 -PS/buffer interface (in the absence of adsorbed biomolecules) shows one large continuum of weak intensity centered around 3100 cm^{-1} .¹⁷ The water structure is not perturbed by the presence of adsorbed L-lysine, despite the high solution concentration of amino acid. This is in contrast to poly-L-lysine which slightly increased the water signal. In the case of poly-L-proline, however, the interfacial water signal is greatly enhanced; this is in opposition to the adsorbed L-proline, which showed nearly no change in interfacial water signal upon amino acid adsorption. The physical interpretation of this is that amino acids and peptides can influence the ordering of water molecules at the solid/liquid interface.

The SFG spectrum of poly-L-lysine on SiO_2 is presented in Figure 3 (a) and the SFG spectrum of L-lysine on SiO_2 is presented in Figure 3 (b). One small mode is observed at 2960 cm^{-1} . The assignment of this peak is non-trivial: there is no evidence for a vibrational mode of lysine at this energy. This mode is tentatively assigned to CH_2 (as) that is strongly perturbed due to a strong electrostatic interaction between the positively

charged amine groups on the lysine side-chains and the negatively charged surface of the silica. The interfacial SFG spectra of poly-L-proline and L-proline at the SiO₂/buffer interface are shown in figures 4 (a) and (b), respectively. The high concentration L-proline may show a small mode around 3000 cm⁻¹, attributed to C_γH₂ (as) and C_βH₂ (as), possibly shifted due to interactions with the highly charged surface.

On SiO₂, the water signal is much more intense, showing two broad modes centered around ~3200 cm⁻¹ and ~3400 cm⁻¹. These can be attributed to ice-like tetrahedrally coordinated and water-like less than tetrahedrally coordinated hydrogen bonded water molecules, respectively.^{18,28} Interestingly, the water signal is greatly enhanced when L-lysine is adsorbed to the silica surface; this is in contrast to poly-L-lysine, which shows a decrease in the water signal upon adsorption. The origin of this behavior is not clear: electrostatic effects should be minimized by the high ionic strength of the solution. Both poly-L-proline and L-proline show a decrease in overall water signal intensity upon biomolecule adsorption. Additionally, the SFG water signal at the SiO₂/buffer interface decreases more significantly from background levels upon the introduction of L-proline than from poly-L-proline. Again, the physical interpretation of this phenomenon is that amino acids and peptides can influence the ordering of water molecules at the solid/liquid interface in a nontrivial manner.

QCM. Biomolecule surface concentrations were directly determined from QCM-D measurements assuming the Sauerbrey equation is valid.²⁶ It was found that on both the hydrophobic d₈-PS/buffer and the hydrophilic SiO₂/buffer interfaces poly-L-lysine adsorbs to a greater extent than its constituent amino acid L-lysine. However, the opposite trend is observed in the case of poly-L-proline which sticks to both surfaces less

extensively than its corresponding amino acid, L-proline. Raw 3rd harmonic QCM-D frequency and dissipation data for poly-L-proline on SiO₂ is presented in Figure 5 as a representative example of all QCM-D measurements in this study. A summary of QCM-D results are included in Figure 6 and stated in Table I.

Discussion

The broad range of biomolecule solubilities as well as SFG responses for the four chemical species studied here required that QCM-D and SFG experiments be conducted over a range of solution concentrations spanning 0.5 mg/mL and 500 mg/mL in the cases of poly-L-proline and L-proline, respectively. Although the experimental data can not determine whether a monolayer, well organized multilayers, or laterally aggregated clumps exist on the surface, it is certainly the case that the bulk concentrations used were high enough for the entire surface to be covered with adsorbate based solely on geometric arguments. Therefore, we interpret these results presented here as being free of the constraints imposed by analyte availability for surface adsorption.

Although the interfacial concentration of L-proline determined by QCM-D measurements is much greater than the interfacial concentration of poly-L-proline, the SFG signal intensity at the *d*₈-PS/solution interface is weaker by approximately one order of magnitude. This is somewhat surprising as the SFG intensity, $I_{SFG} \propto N^2$, where N is the surface number density.²⁹ Therefore, this result can only be interpreted in one way: L-proline is much less ordered than poly-L-proline at the *d*₈-PS/solution interface.¹⁷ The physical origin of this effect is not clear from the experiments described here, but we postulate that the decreased interfacial ordering of L-proline (vs. poly-L-proline) at this interface is due to the weak interactions between the adsorbate and the surface. That is,

the driving force for adsorption here is probably not due to direct (i.e. electrostatic or van der Waals) forces between the adsorbate and surface, but rather driven by the rearrangement of solvent molecules (water) around both the adsorbate and surface.^{30,31} In the absence of strong forces, L-proline is likely to adsorb in many conformations, and thus appear disordered and give weak SFG signals. Poly-L-proline, on the other hand, has many less degrees of freedom and will adsorb in fewer conformations on the surface.

The case of L-lysine and poly-L-lysine at the d_8 -PS/buffer interface is somewhat different. Here, the SFG intensity of the poly-L-lysine is also greater than the corresponding amino acid and in this case by a factor of approximately ten. Here, however, the interfacial concentration of poly-L-lysine measured by QCM-D is higher than L-lysine, meaning that the stronger signal of poly-L-lysine could be due to higher interfacial concentration or more ordering (the solubility limit of L-lysine is much less than L-proline, and hence we were unable to perform experiments at very high solution concentrations of L-lysine). Nonetheless, we hypothesize that the driving force for adsorption onto a hydrophobic surface such as polystyrene will be similar for lysine (and poly-L-lysine) and proline (and poly-L-proline). Given the fact that the surface concentration of L-lysine is only approximately a factor of two less than the surface concentration of poly-L-lysine, we attribute the smaller SFG signal from L-lysine (relative to poly-L-lysine) to reduced interfacial ordering, in addition to reduced surface concentration.

SFG signal of these molecules at the SiO_2 /buffer interface is much lower than the d_8 -PS/buffer interface.¹¹ Given that the measured interfacial concentrations of the molecules were not substantially different between the two interfaces, the cause of the

reduced signal with the SiO₂ substrate must be due to reduced ordering at this interface (relative to the *d*₈-PS/buffer interface). While the molecular level details of why there is little orientational ordering of adsorbates at this interface remains unclear, we can postulate a possible explanation. The driving force for adsorption at this interface should be different than the *d*₈-PS/buffer interface; namely, SiO₂ is negatively charged at pH 7.4 where these experiments were conducted. These short range, relatively strong electrostatic interactions could possibly trap adsorbates in the configuration they adsorb in.^{32,33} That is, unlike the thermodynamic arguments given in the previous two paragraphs, we believe kinetics control the lack of interfacial order of these adsorbates. It is interesting to note that Paszti et al.¹⁵ also did not observe *in-situ* SFG signal of amino acids from the SiO₂/aqueous interface, although their experimental conditions were slightly different (they examined aspartic acid, glutamic acid, glutamine, cysteine, and phenylalanine at lower pHs than this work, and in $P_{\text{SFG}}P_{\text{VIS}}P_{\text{IR}}$ polarization combination).

The structure of water at interfaces remains an active area of research¹⁸ and debate.^{34,35} The largely featureless SFG spectrum of the *d*₈-PS/ buffer interface contains only a small, broad signal from coordinated O-H modes between 3000-3200 cm⁻¹. Using the nomenclature for the SiO₂/water and air/water interfaces, we assign this peak to “ice-like” water. By this assignment, we do not mean to imply the chemical nature of water at the *d*₈-PS/buffer interface is identical to “ice like” water at the SiO₂/water interface. The spectrum contains no free O-H mode⁵ and little intensity around ~3400 cm⁻¹; furthermore, sensitivity to contamination³⁶ makes understanding water structure at the *d*₈-PS/buffer interface difficult. Certainly, water structure present at the *d*₈-PS/buffer interface is quite different than “model” hydrophobic surfaces, such as the air/water³⁷ and

OTS/water interfaces.³⁸ The SFG spectrum of the d_8 -PS/buffer interface is quite similar to the dichloromethane/water interface that has been studied by Richmond and co-workers.^{39,40} They determined that the water structure is a consequence of increased water penetration in the organic phase (something that is not likely here)⁴⁰ and general disorder at the interface.³⁹

The poly-L-lysine and L-lysine results presented here are in stark contrast to our previous studies on amphiphilic peptides adsorbed on hydrophobic and hydrophilic surfaces.^{5,10,17,41} There, on hydrophobic d_8 -PS, the hydrophobic portion of the peptide was ordered and observed in the SFG spectra.¹⁰ Here, we see the opposite: hydrophilic L-lysine and poly-L-lysine having ordered hydrocarbon chains on d_8 -PS.

We have previously shown that an N-H mode is observed in SFG spectrum of the LK₁₄, a 14-residue α -helical amphiphilic peptide composed of L-lysine and L-leucine subunits, on a hydrophilic surface.¹⁷ This mode was attributed to either the backbone Amide A of an α -helix or the terminal amine on the hydrophilic, positively charged lysine side chains. This mode is not obviously apparent in the SFG spectra of L-lysine, poly-L-lysine, poly-L-proline, or L-proline on SiO₂. In fact, a C-H mode is observed in the SFG spectrum of poly-L-lysine and L-proline on SiO₂. These results demonstrate that the ordered parts of adsorbed peptides are strongly dependent on the amphiphilicity, or more generally, the chemical nature of the peptide.

QCM-D showed a greater mass of proline adsorbed than poly-L-proline on both surfaces studied. This could be a result of solvated individual L-proline species at this pH being zwitterionic with a neutral charge balance thus enabling the formation of stacked layers on the surface because of electrostatics. The poly-L-proline does not have this

electrostatic driving force because the amine and acidic portions of each proline unit are replaced with amide bonds along the backbone leaving primarily only weaker intermolecular forces to drive adsorption and cohesion at the interface. In the case of lysine QCM-D experiments indicated that its homopeptide, poly-L-lysine, adsorbed more extensively than the amino acid on both the d_8 -PS and the SiO_2 liquid solid interfaces. This phenomenon may be explained by both the amino acid and the homopeptide having positively charged side chains in solution at pH 7.4. As a result, both the amino acid and homopeptide molecules of lysine are expected to experience an increasing number of repulsive interactions between the positively charged side chains of neighboring species on the surface with increasing surface concentration. However, due to the molecular mass of poly-L-lysine species being orders of magnitude greater than that of an individual amino acid it is reasonable to expect a larger mass of the homopeptide would be bound to the surface of the same area. While the above arguments could rationalize the results on a molecular level, it is important to keep in mind that the solution concentration of poly-L-proline was three orders of magnitude smaller than L-proline, and the solution concentration of poly-L-lysine and L-lysine were nearly identical. Experiments to understand how solution concentration influences interfacial ordering and amount adsorbed are currently under way in our laboratory.

Conclusions

We have demonstrated both the feasibility of studying amino acids via SFG vibrational spectroscopy at the solid/liquid interface as well as using this technique to investigate amino acids and peptides at the silica/water interface. Our results demonstrate that ordering of lysine and proline amino acid side chains occur at the d_8 -PS/buffer

interface. Additionally, we have observed ordering of poly-L-lysine side chains at the silica/buffer interface. On the hydrophobic surface longer peptides showed more SFG signal attributed to the adsorbed species than did their substituent amino acids. We interpret this as increased ordering among longer peptides at the hydrophobic liquid-solid interface. On the hydrophilic surface no clear trend was observed correlating SFG signal intensity and biomolecule chain length. These results at both hydrophobic and hydrophilic surfaces are significantly different than our previous studies of adsorbed amphiphilic peptides. Finally, QCM-D showed that the extent to which the homopeptides adsorb relative to their corresponding amino acids may significantly depend upon side chain polarity and hydrophobicity in addition to conventional factors including molecular mass and solution concentration.

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Figure and Table Captions

Figure 1. (a) The SFG spectra of the PBS buffer/ d_8 -PS interface (open black squares) and the poly-L-lysine/ d_8 -PS interface (red circles). The concentration of poly-L-lysine was 12.5 mg/mL. The broad peak centered on $\sim 3100\text{ cm}^{-1}$ is attributed to interfacial water. Upon adsorption, two intense peaks are seen at 2870 cm^{-1} and 2935 cm^{-1} . These two modes are assigned to CH_2 (s) and CH_2 (as) respectively. The SFG intensity in the water region is slightly increased in the presence of adsorbed poly-L-lysine. (b) The SFG spectra of the PBS buffer/ d_8 -PS interface (open black squares) and the L-lysine/ d_8 -PS interface (red circles). The concentration of L-lysine was 16.5 mg/mL. The broad peak centered on $\sim 3100\text{ cm}^{-1}$ is attributed to interfacial water. Upon adsorption of L-lysine, the water structure is not perturbed but two modes of smaller intensity are observed at 2870 cm^{-1} and 2935 cm^{-1} . These modes can be assigned to CH_2 (s) and CH_2 (as), respectively.

Figure 2. (a) The SFG spectra of the PBS buffer/ d_8 -PS interface (open black squares) and the poly-L-proline/ d_8 -PS interface (red circles). The concentration of poly-L-proline was 0.5 mg/mL. The broad peak centered on $\sim 3100\text{ cm}^{-1}$ is attributed to interfacial water, and increases upon peptide adsorption. Three intense peaks are seen at 2875 cm^{-1} , 2935 cm^{-1} , and 2980 cm^{-1} . These three modes are assigned to a combination of $\text{C}_\delta\text{H}_2$ (s), C_βH_2 (s), and $\text{C}_\gamma\text{H}_2$ (s); a combination of $\text{C}_\gamma\text{H}_2$ (s) and C_βH_2 (s); and a combination of $\text{C}_\gamma\text{H}_2$ (as) and C_βH_2 (as), respectively. The inset shows proline amino acid with labeled carbons. (b) The SFG spectra of the PBS buffer/ d_8 -PS interface (open black squares) and the L-proline/ d_8 -PS interface (red circles). The concentration of L-proline was 500 mg/mL. The broad peak centered on $\sim 3100\text{ cm}^{-1}$ is attributed to interfacial water, and is constant with amino acid adsorption. Three peaks are seen at 2875 cm^{-1} , 2935 cm^{-1} , and 2980 cm^{-1} . These

three modes are assigned to a combination of $C_{\beta}H_2$ (s), $C_{\gamma}H_2$ (s), and $C_{\delta}H_2$ (s); a combination of $C_{\beta}H_2$ (s) and $C_{\gamma}H_2$ (s); and a combination of $C_{\beta}H_2$ (as) and $C_{\gamma}H_2$ (as), respectively.

Figure 3. (a) The SFG spectra of the PBS buffer/silica interface (open black squares) and the poly-L-lysine/silica interface (red squares). The concentration of poly-L-lysine was 12.5 mg/mL. The buffer/silica interface water structure shows two very large peaks around $\sim 3200\text{ cm}^{-1}$ and $\sim 3400\text{ cm}^{-1}$, attributed to tetrahedrally and less than tetrahedrally coordinated hydrogen bonded water, respectively. Upon adsorption of poly-L-lysine, the overall SFG intensity in the water region is reduced, especially around 3400 cm^{-1} . The C-H mode seen around $\sim 2960\text{ cm}^{-1}$ is attributed the methylene groups of the adsorbed poly-L-lysine. (b) The SFG of the PBS buffer/silica interface (open black squares) and the L-lysine/silica interface (red circles). The solution concentration of L-lysine is 16.5 mg/mL. The water structure shows two very large peaks around $\sim 3200\text{ cm}^{-1}$ and $\sim 3400\text{ cm}^{-1}$, attributed to tetrahedrally and less than tetrahedrally coordinated hydrogen bonded water, respectively. Note the increase in water signal upon L-lysine adsorption.

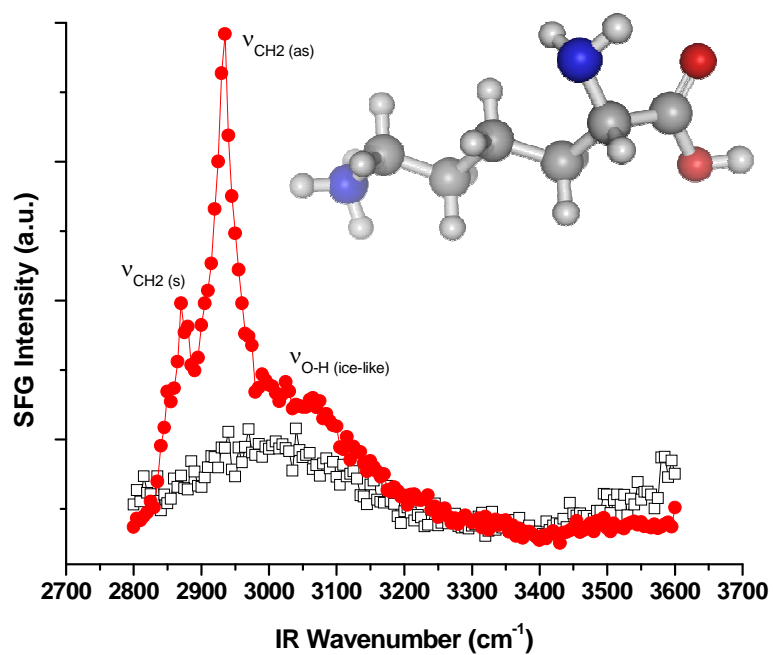
Figure 4. (a) The SFG spectra of the PBS buffer/silica interface (open black squares) and the poly-L-proline/silica interface (red circles). The solution concentration of poly-L-proline is 0.5 mg/mL. The water structure shows two very broad peaks around $\sim 3200\text{ cm}^{-1}$ and $\sim 3400\text{ cm}^{-1}$, attributed to tetrahedrally and less than tetrahedrally coordinated hydrogen bonded water, respectively. There is little change in the water signal upon poly-L-proline adsorption. (b) The SFG spectra of the PBS buffer/silica interface (open black squares) and the L-proline/silica interface (red circles). The solution concentration of L-proline is 500 mg/mL. The water structure shows two very broad peaks around ~ 3200

cm^{-1} and $\sim 3400 \text{ cm}^{-1}$, attributed to tetrahedrally and less than tetrahedrally coordinated hydrogen bonded water, respectively. There is a decrease in the water signal upon L-proline adsorption.

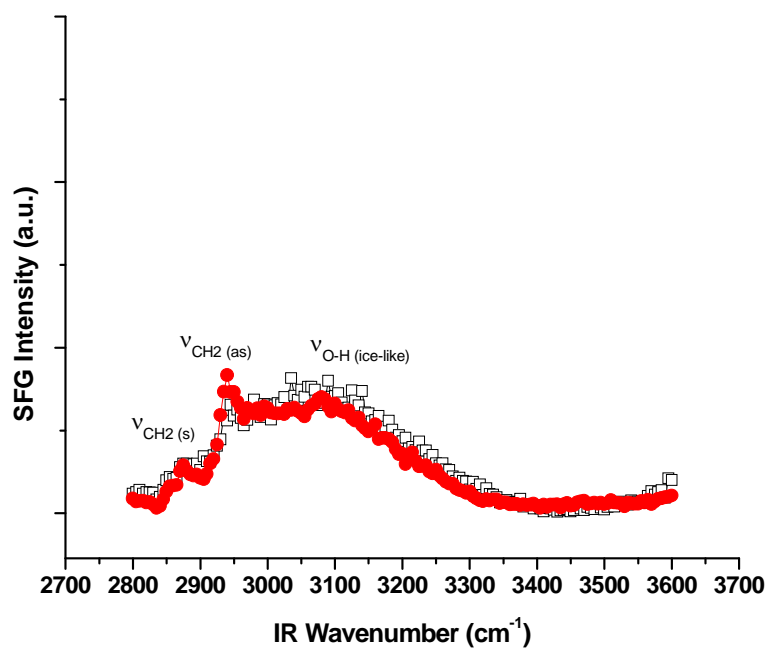
Figure 5. Raw QCM-D data for frequency (red squares) and dissipation (blue circles) measurement of the 3rd harmonic of a SiO_2 coated sensor crystal in PBS buffer before, during, and after being exposed to poly-L-lysine solution.

Table I. Surface concentration of the molecules examined in this study, determined by QCM.

Figure 6. Surface concentration of the molecules examined in this study, determined by QCM.

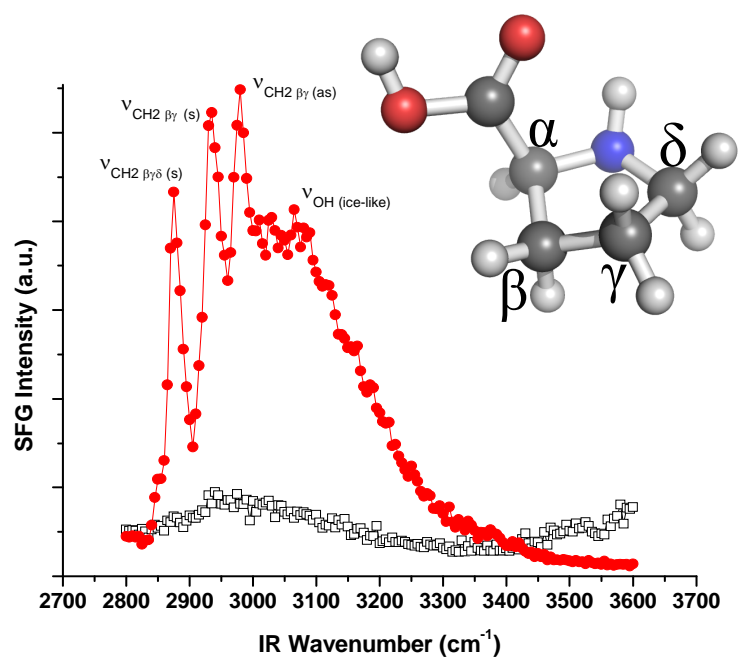


(a)

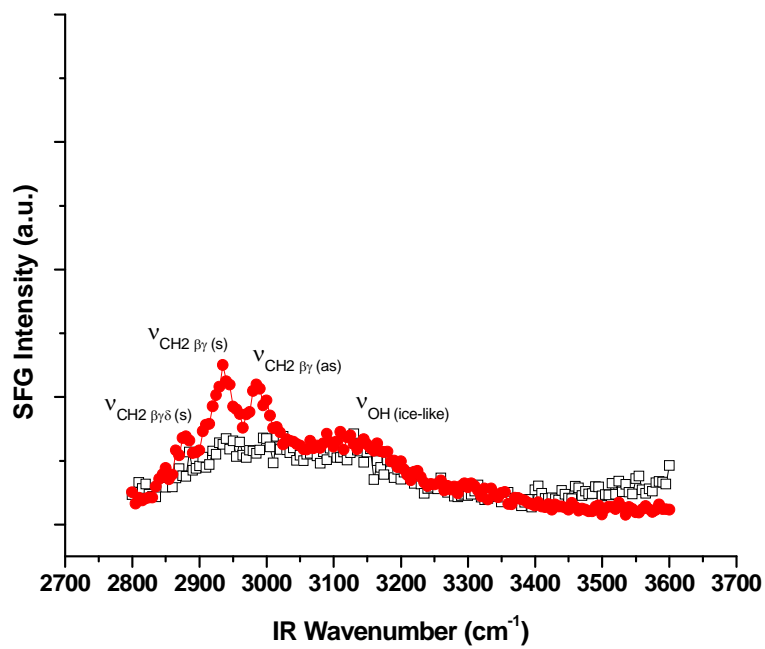


(b)

Figure 1.

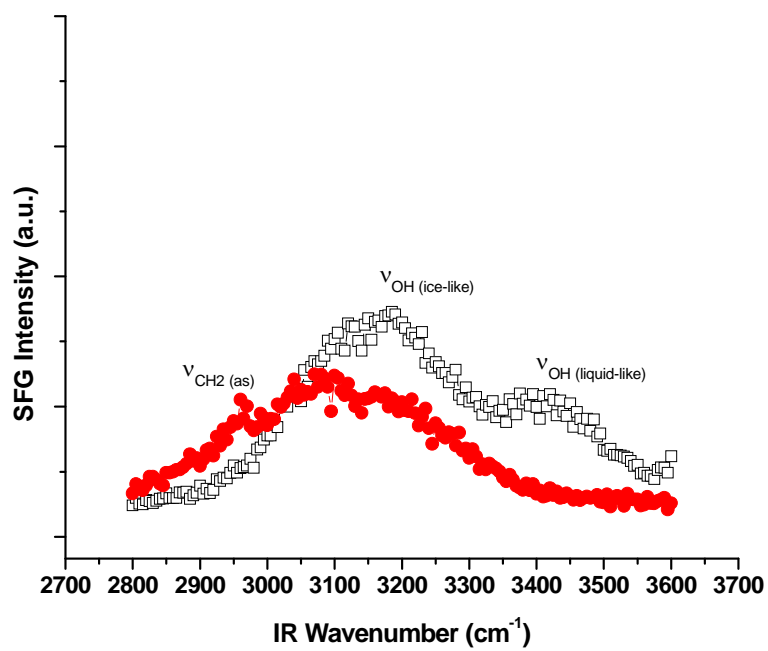


(a)

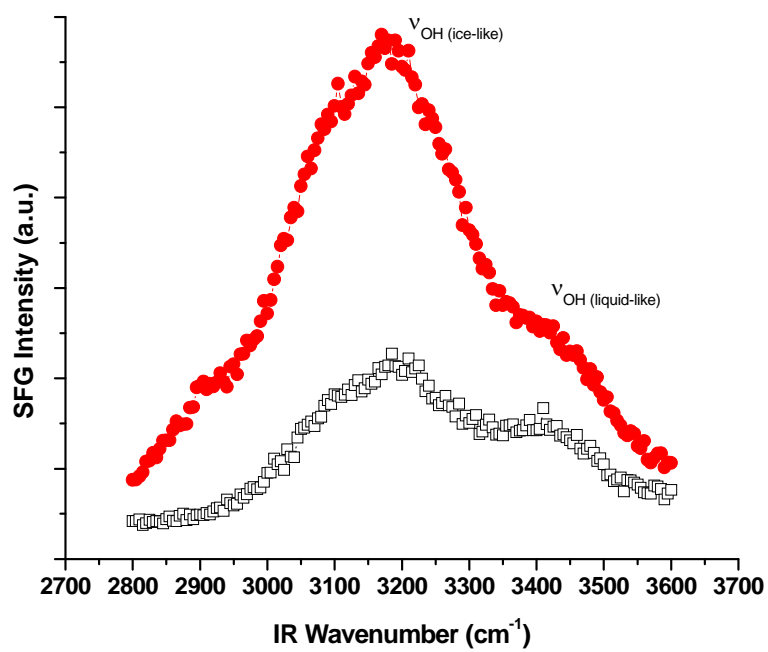


(b)

Figure 2.

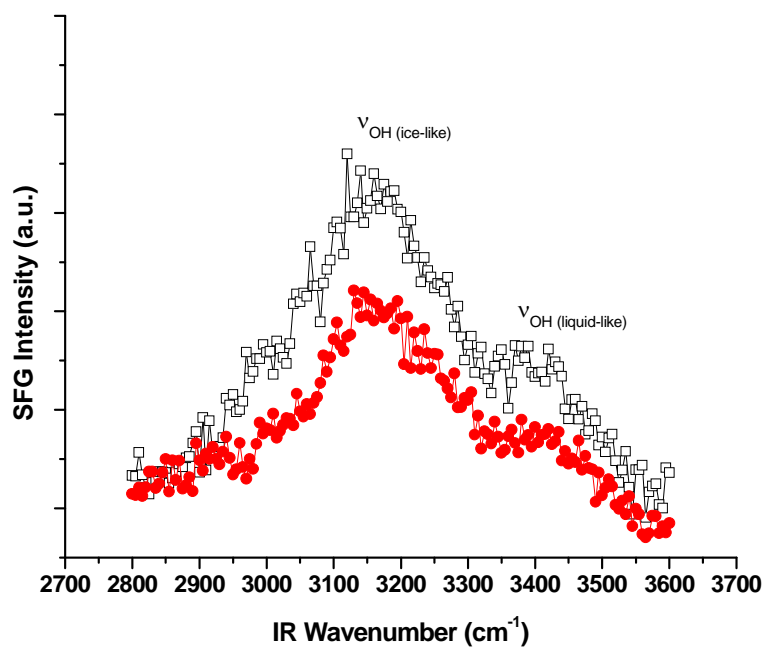


(a)

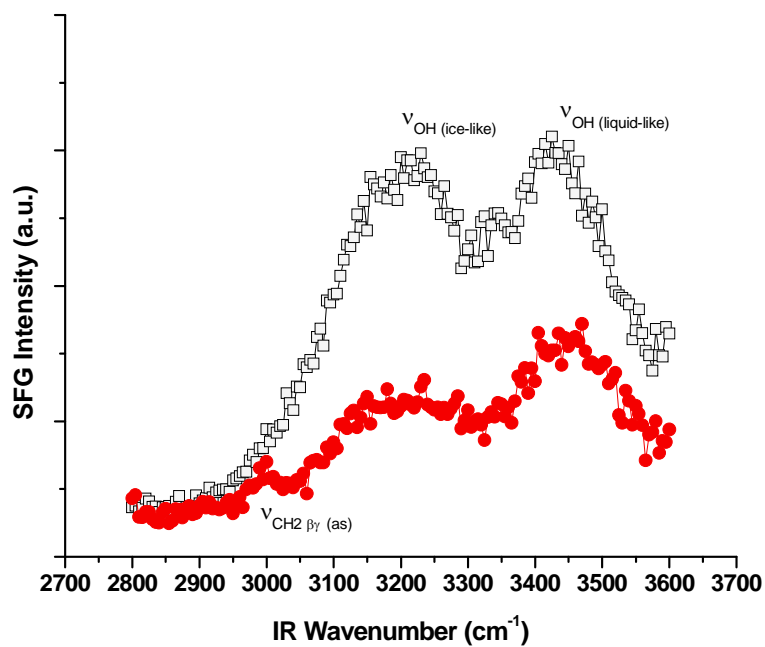


(b)

Figure 3.



(a)



(b)

Figure 4.

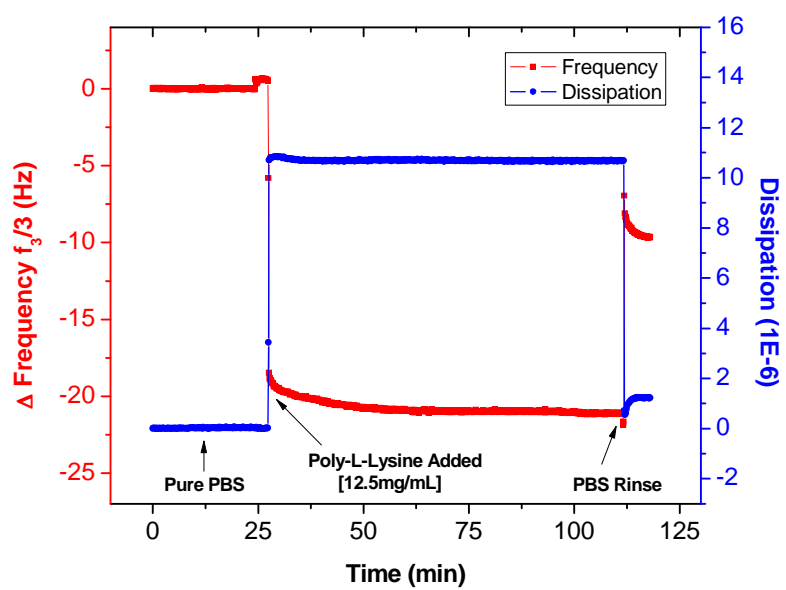


Figure 5.

Species (Solution Conc.)	<i>d</i> ₈ -Polystyrene Surface Concentration	SiO ₂ Surface Concentration
L-Lysine (16.5 mg/mL)	243 ± 25 ng/cm ⁻²	248 ± 23 ng/cm ⁻²
Poly-L-Lysine (12.5 mg/mL)	457 ± 42 ng/cm ⁻²	365 ± 9 ng/cm ⁻²
L-Proline (500 mg/mL)	5770 ± 60 ng/cm ⁻²	6070 ± 130 ng/cm ⁻²
Poly-L-Proline (0.5 mg/mL)	140 ± 31 ng/cm ⁻²	171 ± 29 ng/cm ⁻²

Table I.

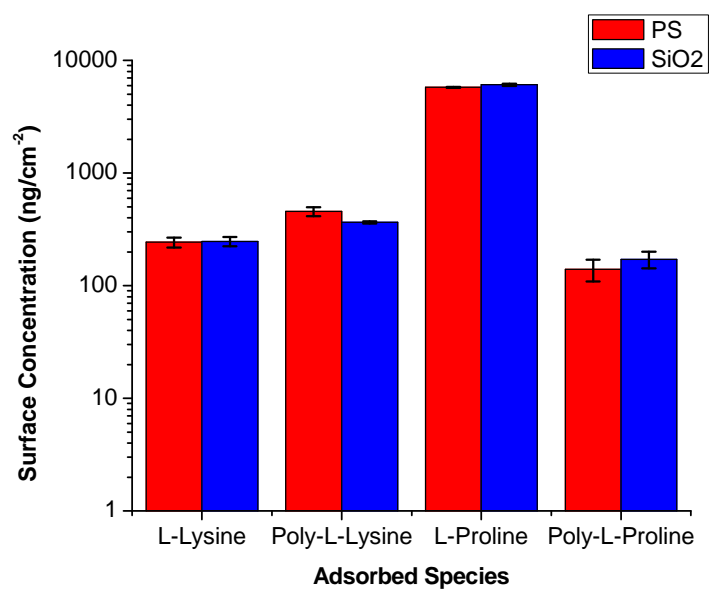


Figure 6.

TOC Graphic

